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14. ABSTRACT

My proposal is designed to discover the role of DLC1 in the action of tamoxifen in breast cancer and the involvement of its phosphorylation by Pak1 in this context. The hypothesis is that tamoxifen stimulates the expression and function of DLC1, and that deregulation of DLC1 stimulates the expression of ER-target genes and leading to an enhanced cell survival, and inability of tamoxifen to suppress the action of estrogen in breast cancer cells. We plan to investigate the following points. (1) Study the mechanisms of tamoxifen regulation of DLC1 expression in breast cancer cells. (2) Determine the functional consequences of tamoxifen regulation of DLC1 expression upon the biology of breast cancer cells. (3) Define the effects of DLC1-WT and DLC1-Ser-88-Ala mutant in a transgenic murine model. To date, we found that overexpression of DLC1 caused mammary alveolar hyperplasia, an early stage of mammary neoplasia. Moreover, mammary glands from MMTV-DLC1-Ser88Ala mice had accelerated involution. Together, these results suggest that deregulated DLC1 could alter mammary gland development and its phosphorylation by Pak1 is essential for its function.

15. SUBJECT TERMS
Breast cancer, Dynein light chain 1, P21-activated kinase 1, transgenic mice model, tamoxifen

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Introduction

Tamoxifen is known to be effective in breast cancer treatment, however, many patients do not respond or develop resistance to it. Thus far, the mechanisms underlining tamoxifen resistance is not very clear. P21-activated kinase 1 (Pak1), a serine/threonine kinase which plays multiple roles in human cancers, has been shown to be associated with tamoxifen resistance in breast cancer patients (1-3). Dynein light chain 1 (DLC1), a substrate of Pak1, is a downstream effector of estrogen and coactivator of ER pathway (4,5). Furthermore, Pak1 phosphorylation of DLC1 has been shown to be essential for DLC1's function in breast cancer (4). The purpose of this study is to determine the influence of DLC1 upon the sensitivity of the breast cancer cells to tamoxifen and whether its phosphorylation by Pak1 is required for its function in tamoxifen resistance. The accomplishments from this study will provide insights to understanding mechanisms of breast cancer resistance to tamoxifen and to new therapeutic designs of breast cancer treatment.

Body

A. Specific Aims:

(1) Study the mechanisms of tamoxifen regulation of DLC1 expression in breast cancer cells.
(2) Determine the functional consequences of tamoxifen regulation of DLC1 expression upon the biology of breast cancer cells.
(3) Define the effects of DLC1-WT and DLC1-Ser-88-Ala mutant in a transgenic murine model.

B. Studies and results:

In the past year, we followed the proposal in the statement of work and focused on aim 3 to investigate DLC1's role in breast cancer and Pak1 phosphorylation effect on DLC1's function in MMTV-DLC1 and MMTV-DLC1-Ser88Ala mice models. The details of the progress will be discussed as following.

<u>Overexpression of DLC1 gene led to alveolar hyperplasia in multiparous mice</u>

Previous studies suggested that DLC1 promotes breast cancerous phenotypes (4). To examine whether DLC1 is able to initiate breast tumor in whole animal setting, we observed a coherent of 25 –30 multiparous mice from each group of WT, MMTV-SLC1 and MMTV-S88A mice as these mice aged from 6 months to 16-18 months old. The palpable mammary tumor incidence in

every group was rare. WT mice developed one adenocarcinoma, MMTV-DLC1 mice developed one carcinoma and one fibrosarcoma, and MMTV-S88A mice developed two fibrosarcoma. To further examine whether overexpression of DLC1 is involved in the early steps of mammary tumorigenesis in vivo, we dissected the mammary glands from some of these 16-18 months old female mice. Whole mount and histological analysis of the glands revealed the presence of mammary alveolar hyperplasia (Figure 1). The incidence of mammary alveolar hyperplasia was about 9% in WT mice and zero in MMTV-S88A mice, whereas the ratio was signicantly increased to about 60% in MMTV-DLC1 mice (Figure 2).

MMTV-S88A mice exhibited accelerated mammary involution

Because of the key role of apoptosis in the mammary involution and the potential function of DLC1 in the mammary apoptosis, we next studied the MMTV-DLC1 and MMTV-S88A mammary development during involution. The #4 inguinal mammary glands from involution time day 1 to day 5 were dissected and analyzed by H&E. By 24 hours after removing the pups, mammary glands from both MMTV-DLC1 and MMTV-S88A mice showed no difference with wild type mice. The alveoli were expanded and surrounded with single-layered epithelial cells. On involution day 2, alveoli of the mammary glands started to regress and adipocyte cells began to repopulate. This process was more distinct in mammary glands from MMTV-S88A mice. Furthermore, some alveoli of the mammary glands from MMTV-S88A mice had collapsed. On involution day 3, the majority of lobuloalveolar structures collapsed and formed clusters of epithelial cords with small lumina. Ducts appeared and fat cells were obvious. Mammary glands from MMTV-DLC1 mice showed similar phenotype as the WT mice, whereas mammary glands from MMTV-S88A mice had accelerated involution progression with the regression of epithelial component and reemergence of adipocytes to a further extent. The accelerated involution of the mammary glands from MMTV-S88A mice lasted to involution day 4 (Figure 3). By day 5 of involution, there was no much difference among the mammary glands from these mice.

To assess the differences in the extent of mammary involution among the WT, MMTV-SLC1 and MMTV-S88A mice, we quantified the portion of gland occupied by fat cells which has been used as a mammary involution index. Mammary glands from transgenic mice had similar percentage of fat cells on day 2 as that from the wild type mice. However, the proportional

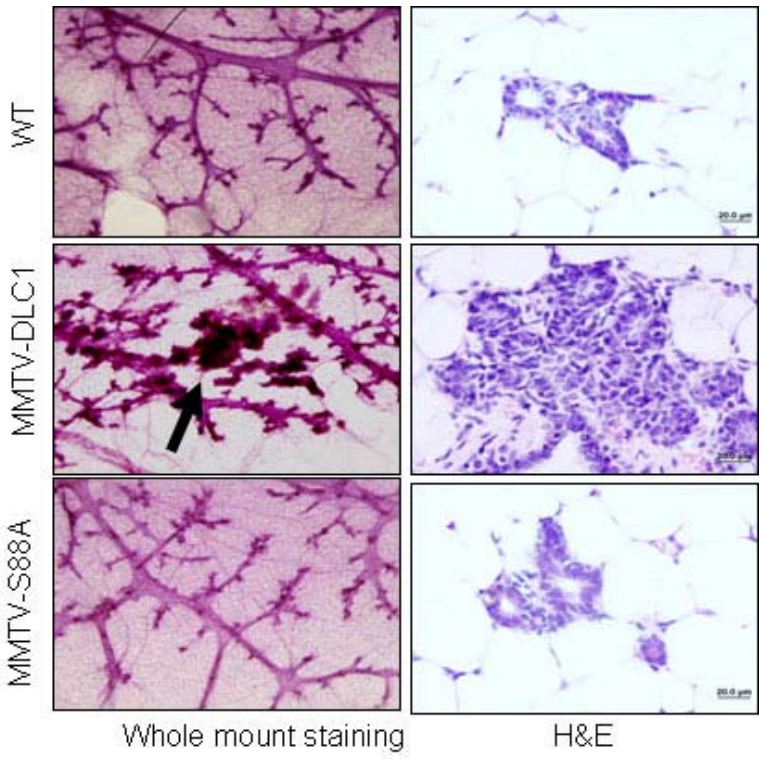

Fig. 1. Mammary glands from multiple-parous WT, MMTV-DLC1 and MMTV-S88A mice about 16 to 18 months of age were analyzed by whole-mount and histologic analysis. The whole mount was stained with carmine solution and histologic sections were stained with hemotoxyline & eosin. The arrow is referring to alveolar hyperplasia. Bars, 500 μm in whole-mount and 20 μm in hematoxylin and eosin staining.

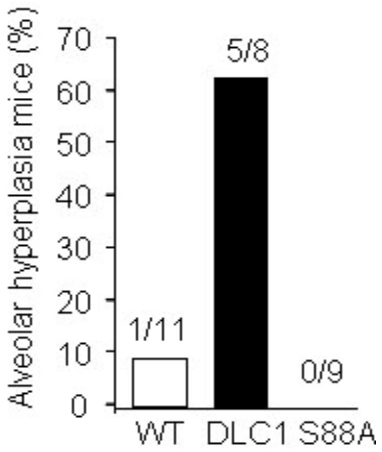

Fig. 2. The number of WT, MMTV-DLC1 and MMTV-S88A mice that developed alveolar hyperplasia was determined and the values were expressed as the percentage of alveolar mice out of total mice examined.

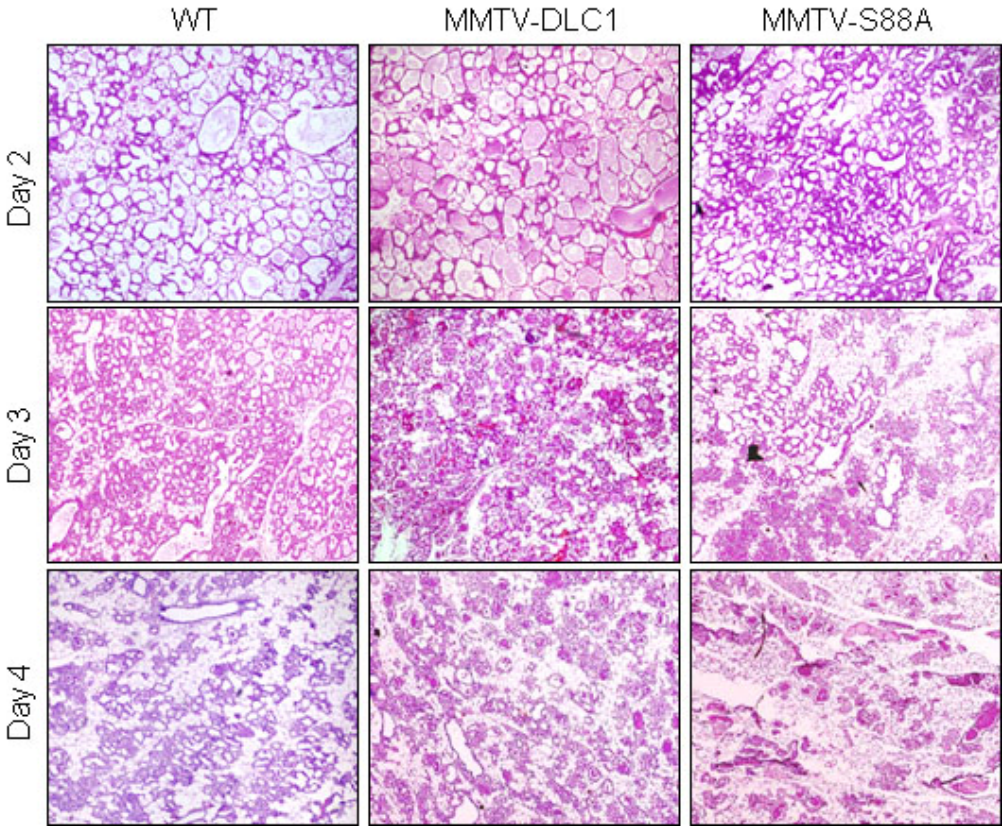

Fig. 3. The mammary involution was synchronized by removing the pups on day 10 lactation. Representative hemotoxylene & eosin staining of mammary glands from WT, MMTV-DLC1 and MMTV-S88A mice during involution at day 2, 3 and 4 time points were shown.

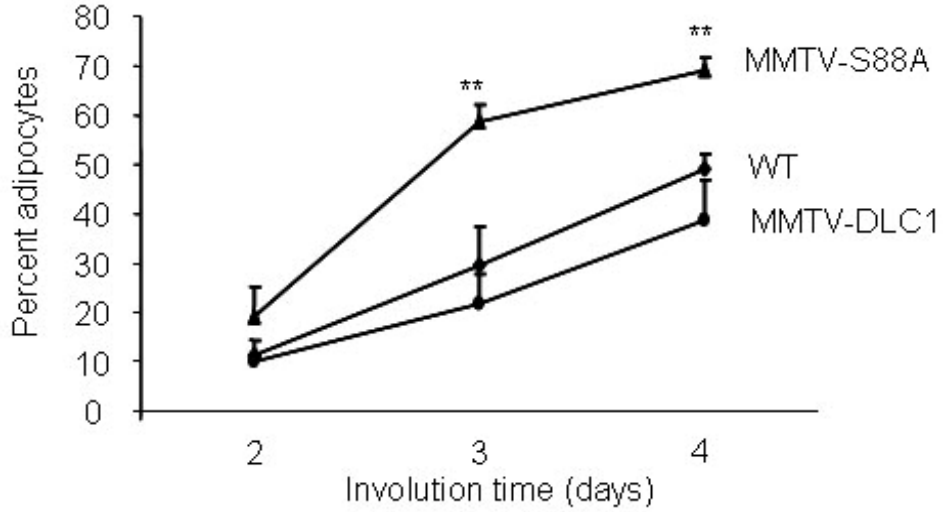

Fig. 4. Quantification of adipocytes content of WT, MMTV-DLC1 and MMTV-S88A mammary glands during involution from the indicated time points (three mice per time point). The values are expressed in percentage of area filled with adipocytes in five random selected fields in the mammary gland.

increase in adipose tissue was significantly accelerated in MMTV-S88 mice on involution day 3 and day 4 (Figure 4). Taken together, these findings suggested overexpression of wild type DLC1 didn't alter mammary involution. However, DLC1-S88A might be able to interfere the survival function of endogenous DLC1 in vivo since S88A transgenic mice had faster mammary involution.

Key research accomplishments

(1) We analyzed the phenotypic alteration of mammary glands from MMTV-DLC1 and MMTV-DLC1-Ser88Ala transgenic mice at virgin, pregnancy, lacation and involution stages
(2) We found that overexpression of DLC1 could cause mammary gland alveolar hyperplasia and Ser88 phosphorylation is essential for this
(3) Mammary glands from MMTV-DLC1-Ser88Ala mice showed faster involution, which implicated phosphorylation could regulate DLC1's function on cell survival

Reportable outcomes

Support from this fellowship has allowed the PI to complete her Ph.D. dissertation work, leading to Ph.D. degree in early 2007 (Ph.D. public defense finished on Dec 08, 2006).

One manuscript is being prepared with the acknowledgement of this grant.
Chunying Song, Rakesh Kumar. Ser88 phosphorylation regulates dynein light chain 1 (DLC1) function in the mouse mammary gland.

Poster presentation at 97th AACR, 2006.
Chunying Song, Hao Zhang and Rakesh Kumar. Dynein light chain 1 in the mouse mammary gland.

Conclusions

Pak1 has multiple functions in the normal development and cancer. More importantly, its expression and subcellular location was shown associated with tamoxifen resistance in human breast cancer. DLC1, one of the targets of Pak1, might be one mediator of Pak1's role in tamoxifen resistance since DLC1 could enhance estrogen receptor activities. In the preliminary results of this proposal, we have shown that tamoxifen could increase DLC1 expression and the overexpression of DLC1 was able to elevate tamoxifen resistance in human breast cancer cells.

In addition, mammary glands from MMTV-DLC1 mice, but not MMTV-DLC1-Ser88Ala mice, showed hyperbranching and elevated cell proliferation. In the past year, we further analyzed the mice and found that overexpression of DLC1 caused mammary alveolar hyperplasia, an early stage of mammary neoplasia. Moreover, mammary glands from MMTV-DLC1-Ser88Ala mice had accelerated involution. Together, these results suggest that deregulated DLC1 could alter mammary gland development and its phosphorylation by Pak1 is essential for its function. To directly test the role of transgene expression in the action of tamoxifen, I will determine the effect of implantation of tamoxifen pellet on the potentiation of the above biological effects. I expect that tamoxifen may not have any effect in the wild-type animal but could promote hyper-stimulatory responses in TG animals. Further, if Pak1 phosphorylation site on DLC1 is important for putative effects of tamoxifen, then I may not observe any such change in DLC1-Ser88Ala mice.

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